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Proper Developmental Control of Human Globin Genes Reproduced by Transgenic Mice Containing a 160-kb BAC Carrying the Human β-Globin Locus

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ABSTRACT: Four independent bacterial artificial chromosome (BAC) clones containing the human β-globin gene locus were obtained from a human genomic BAC library. A 160-kb clone (186D7) carrying the entire human β-globin locus including the β-globin gene family, locus control region (LCR), and 3′ regulatory elements was used to transform mice. Four transgenic lines were generated by microinjecting the purified BAC DNA into the fertilized eggs. RNase protection analysis showed that the expression of human β-globin genes is tissue- and developmental stage-specific and the expression level is similar among the three independent transgenic lines which carry the entire human β-globin locus; however, no β-globin gene expression was detected in the transgenic mice lacking the LCR region. The results suggest that the transgenic mouse model system that we have produced and that uses BAC to study the complex human β-globin gene cluster is stable and reproducible. Our results also indicate that some newly characterized HSs upstream from the LCR appear not to play an important role in globin gene expression and switching, while the traditional LCR can ensure correct human β-globin gene expression in transgenic mice. The BAC-mediated transgenic system can be used for further studies to determine which kinds of *cis*-acting elements are included in regulating the developmental timing and the level of human β-globin gene expression.

INTRODUCTION

The human β-globin gene cluster spans 70 kb of genomic DNA and consists of five functional genes arranged in the order, 5′-ε-^{G}γ-^{A}γ-δ-β-3′, on the short arm of chromosome 11. The expression of each of these genes is limited to erythroid tissues and to a specific developmental stage: the β-globin gene is expressed in the embryonic yolk sac, the ^{G}γ- and ^{A}γ-globin genes are expressed specifically in the fetal liver, whereas the expression of δ- and β-globin genes is detected in the bone marrow in adult (1). Five DNase I-hypersensitive sites (5′HS1–5), located 6–20 kb upstream of the ε-globin gene, are termed the locus control region (LCR) (2). LCR is generally acknowledged to be necessary for the high level expression of globin genes in erythroid tissues (2–4). In addition, another developmentally stable DNase I HS site and some regulatory elements are located in regions downstream from the β-globin gene (5, 6). Thus, sequences spanning a region of over 100 kb have been included in the control of the human β-globin genes.

The human β-globin locus has become a model system for studying the developmental regulation of gene families. Creating transgenic mice carrying the entire human β-globin locus is the main approach. Ligating cosmids (70-kb fragment) and yeast artificial chromosomes (YACs)

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(150- and 248-kb fragments), because of their larger insert size compared with plasmid vector, have been used as the vectors to produce transgenic mice (7–10). Previous results showed that the expression of the transgenic human β-globin genes is tissue- and developmental stage-specific and closely follows the expression pattern of the endogenous mouse β-globin locus. Several research groups have made transgenic lines containing targeted mutations in β-globin YACs or ligated cosmids (11–16). These studies provide us with deep insight about the regulation mechanism of the β-globin gene cluster. As a vector system, cosmids and YACs have limitations. The cosmid, even the ligating cosmid (7, 11), cannot entirely accommodate large gene clusters, including the β-globin gene clusters. The YAC system also has some disadvantages: it is difficult to handle and has a high degree of chimerism and colony instability (17, 18). Some studies showed remarkable disparity in the expression level of exogenous human β-like globin genes among different β-YAC transgenic mice (9).

Though considerable experimental evidences have been accumulated and several models (19, 20) have been proposed for the explanation of expression and switching of the human β-globin gene cluster, many details about the gene cluster are still unclear and some of the experimental results are inconsistent. Previous studies, including transient and stable transfection assays as well as studies in transgenic mice, have revealed isolated components of the LCR and have suggested that the LCR is an element capable of creating a domain that is independent of the influence of flanking chromatin. Groudine and colleagues (21, 22) have recently reported that the LCR is not necessary to keep the locus in an "open" conformation in human erythroid cells or mouse ES cells. Furthermore, the mechanism by which the LCR directs the expression of the β-globin genes remains incompletely defined and the precise function of individual HS sites is also somewhat controversial in different studies (23). Therefore, it is necessary to set up a more stable and reproducible model system in order to study the complex gene cluster. As a new developmental vector system, the bacterial artificial chromosome (BAC) (24) has several advantages which makes it more suitable for the purpose mentioned above. Taking this into consideration, we have developed the BAC system as the transgenic vector of the human β-globin gene cluster. A 160-kb BAC carrying the entire human β-globin locus was characterized and then introduced into the germ line of several mice by microinjection. Tissue- and developmental stage-specific expression of the human locus was observed in the BAC-mediated transgenic mice and the expression levels of human β-globin genes were found to be similar between different transgenic lines.

MATERIALS AND METHODS

Isolation of BACs Containing the Human β-Globin Locus and Restriction Mapping

Three different probes (HS2/*Bgl*II–*Hin*dIII, hβ/*Bam*HI–*Eco*RI, and RK29/*Eco*RI; Fig. 1) were used to screen the human high-density BAC colony membranes (Research Genetics). Positive clones were obtained from the relevant BAC library. BAC DNA miniprep and large-scale preparation were carried out by standard procedures (25). Usually *Not*I (New England BioLabs) digestions of BAC DNA were used to determine the size of the inserted fragments. Restriction fragments were separated using pulse-field gel electrophoresis (PFGE) on 1% MP agarose (B. M.) using the 10- to 350-kb autoprogram of CHEF Mapper (Bio-Rad). Digestions with other restriction enzymes (*Kpn*I, *Sac*I, *Sfi*I, *Hin*dIII, *Eco*RI), Southern blot hybridizations using several human β-globin locus probes, and partial restriction enzyme digestions were performed to further fingerprint the BAC clones.

The probes used to map the BACs are listed below in 5′ to 3′ order with respect to their position in the β-globin region: RI3.3/*Eco*RI (3.3 kb), HS3/*Hin*dIII (1.9 kb), HS2/*Bgl*II–*Hin*dIII (0.7 kb), hε/*Sph*I–*Hin*dIII(3.8 kb), $h^{G}\gamma$/*Sna*BI–*Bam*HI (1.7 kb), $h^{A}\gamma$/*Hin*dIII(3.3 kb), hδ/*Pst*I (2.3 kb), hβ/*Eco*RI–*Bam*HI (0.9 kb), RK29/*Eco*RI (1.2 kb).

About 500 ng BAC DNA was used to sequence directly. Sequencing was done by using dye terminators and universal primers (T7 and

SP6) on an ABI377 automated sequencer (Perkin–Elmer). The sequencing results were compared with published sequences by using Advanced Blast server (http://www.ncbi.nlm.nih.gov/BLAST/) to determine the exact terminal sequence of BAC clones.

Fluorescence in Situ Hybridization Analysis

Human chromosome preparations were made from peripheral blood lymphocytes of a normal male as described previously. About 300 ng BAC DNA probe was labeled with biotin-14-dATP by nick translation (GIBCO-BRL). Hybridization signals were detected with avidin-conjugated fluorescein isothiocyanate (Vector Labs), following two rounds of amplification using biotinylated antiavidin. Hybridization signals were scored using an Olympus BX-60 microscope and images were captured on a photometrics CCD camera using Applied Imaging software.

Generation of Transgenic Mice

Large scale preparation of 186D7 BAC DNA was purified on a cesium chloride gradient ultracentrifugation (75,000 rpm, 5 h). The 160-kb linear *Not*I fragment of BAC 186D7 was purified as described (27). After digestion with *Not*I at 37°C overnight, 50 μg BAC DNA was run through equilibrated CL-4B Sepharose (Pharmacia) columns and 0.5-ml fractions were collected. The fractions containing the intact linear BAC DNA (with minimal vector fragments) were diluted to 0.8–1 μg/ml with injection buffer (10 mM Tris–HCl, pH 7.5, 0.1 mM EDTA, and 100 mM NaCl) and microinjected into the pronucleus of fertilized mouse eggs (from F1 females of mouse strain Km × ICR), which were then transferred into the oviducts of pseudopregnant (mouse strain Km) F1 female mice. Transgenic founders were identified via PCR and Southern blot of tail DNA as previously described (26). PCR primer pairs were as follows: HS5, 5′-GTTCGTCATAATATGGGTTTTT-3′ and 5′-TGTGGGGAAAGAAATTAAATTA-3′; HS2, 5′-CCTCCCATAGTCCAAGCATGAGCAGT-3′ and 5′-GATTCTGCCGCTTCTAGGTATAGAG-3′; humanγ, 5′-GACCGTTTTGGCAATCCATTTC-3′ AND 5′-GTATTGCTTGCAGAATAAAGCC-3′ humanβ, 5′-TACGTAAATACACTTGCAAAGGAGG-3′ and 5′-TTTGAGGTTGCTAGTGAACACAGTT-3′. Positive founders were bred to Km/ICR mice. Genomic DNA (10 μg) from F1 mice was used to determine the copy number by quantitation of Southern blot using PhosphorImager (Molecular Dynamic).

RNase Protection Analysis

Total RNA was prepared from yolk sacs of day 8.5 to 11.5 embryos, livers of day 12.5 to 16.5 fetus, or from different tissues of adult animals using Trizol Reagent (Gibco, RBL) or RNA miniprep kit (Qiagen). RNase protection analysis was performed essentially as described (26). In brief, 10 μg total RNA was hybridized with 1–2 × 10^5 cpm of each probe in solution at 55°C overnight. After hybridization, the samples were digested with ribonuclease A (8 μg/ml) and ribonuclease T1 (10 U/ml) at 25°C for 30 min. The samples were purified and electrophoresed on 4% polyacrylamide/7 M urea gels. Autoradiography was performed and protected bands on the gels were quantitated via PhosphorImage analysis. The following globin-specific probes were used: mouse α,pSP6mα; mouse ζ,pT7mζ; human β, pT7β; human γ, pT7$^A\gamma$; human ϵ, pT7ϵ.

Capillary Electrophoresis (CE)

Blood samples were collected from adult transgenic and nontransgenic mice. Hemoglobin was prepared from peripheral blood as described previously (26). Proper amounts of each sample were separated on CE P/ACE 5500 instrument (Beckman) to determine the human globin expression relying on the different isoelectric focusing point between the endogenous and hybrid hemoglobin tetramers. The cIEF3-10 kit and XIEF programs were used in the experiment.

RESULTS

Isolation and Restriction Mapping of BAC Clones

Four BAC clones containing the human β-globin locus were obtained by screening the

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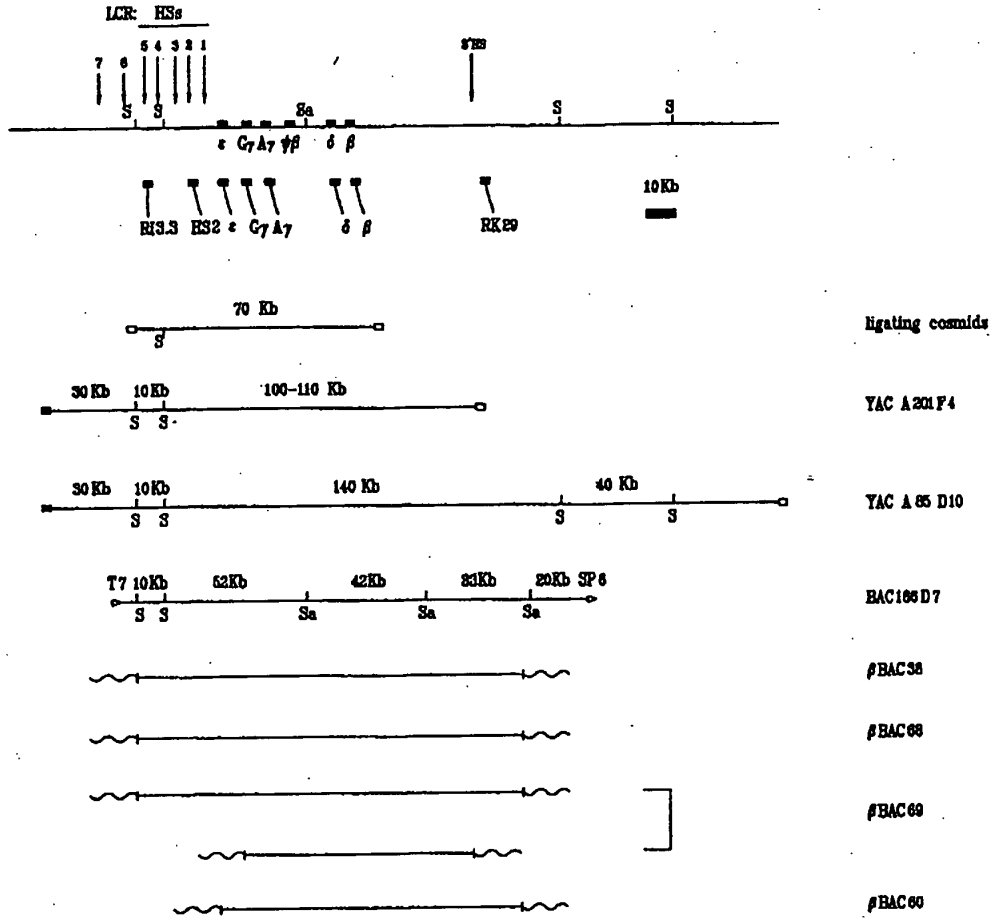

FIG. 1. Physical map of the human globin locus, ligating cosmids, globin YACs A201F4 (150 kb) and A85D10 (230 kb), βglobin BAC186D7 (160 kb), and BAC transgenic lines. The top line indicates the positions of the genes (solid squares) and the HSs (arrows). The restriction sites of *Sal*I (Sa) and *Sfi*I (S) are shown. The positions of probes used to map the human locus in Southern hybridization are represented by the closed boxes. The four lines below it represent the restriction fragment lengths (kb) of the ligating cosmids, YAC A201F4, YAC A85D10, and BAC186D7. The last five lines show the length of BAC186D7 kept in different transgenic mouse lines. Wavy lines at the end represent flanking murine genomic DNA sequences.

BAC library with probes for the genes of the human β-globin cluster. After *Not*I digestion and PFGE of BAC DNA, the approximate size of the four BACs was estimated by comparison with size markers of multimers of bacteriophage λ (New England BioLab) as follows: 118J17 (85 kb), 177P23 (78 kb), 177O23 (78 kb), and 186D7 (160 kb). Except for the 118J17 clone, the three other BACs contained sequences from HS5 to the β gene. However, only the 186D7 BAC DNA hybridized with all the probes tested, and its map is shown in Fig. 1.

To further characterize the structure of the human genomic inserts in the BAC clones, we performed restriction analysis with several rare cutting restriction enzymes. All four BAC clones showed the same *Sfi*I, *Kpn*I, and *Not*I restriction pattern when compared to YAC clones (only data from 186D7 are shown in Fig. 2). Partial digestion of 186D7 BAC DNA with *Sal*I yielded two new bands at about 100 and 140 kb that hybridized with the RI3.3/*Eco*RI probe (Figs. 2C and 2D).

The results of direct BAC DNA sequencing and BLAST search showed that the 5′ end of the

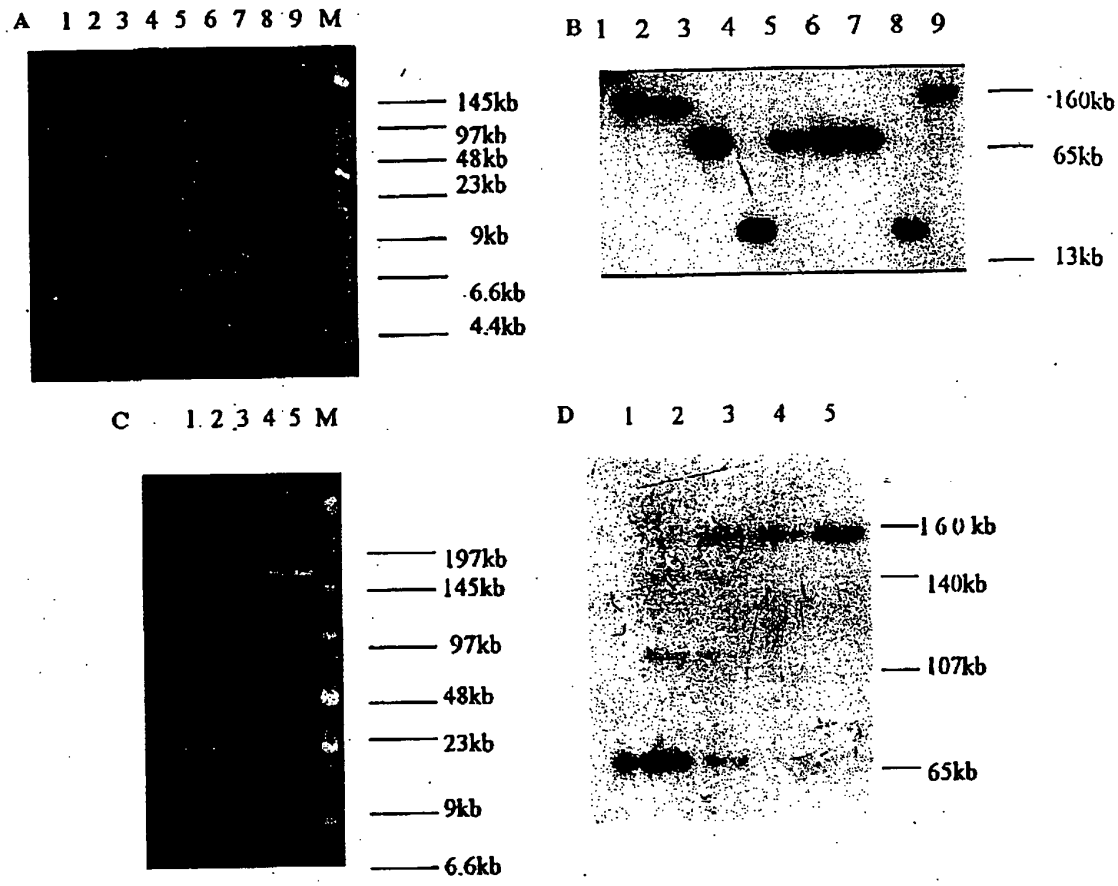

FIG. 2. Pulsed field gel electrophoreses analysis of BAC186D7 clone. (A) Restriction mapping of BAC186D7 DNA. Lane 1, *Not*I; lane 2, *Sfi*I/*Not*I; lanes 3 and 6, *Not*I/*Sal*I; lane 4, *Not*I/*Kpn*I; lane 5, *Sal*I; lane 7, *Sal*I/*Sfi*I; lane 8, *Sal*I/*Kpn*I; lane 9, *Sfi*I; M, low-range PFGE markers (New England Biolabs) (B) Auto radiogram made from the gel described in A. Southern blot was performed with a h probe. Lanes are the same as in A. (C) Partial digestion with SalI of BAC186D7 DNA linearized with *Not*I. Lanes 1–4, partial digests with different concentration of *Sal*I; lane 5, BAC186D7 DNA linearized with *Not*I; M, low-range PFGE markers. (D) Southern analysis of gel described in (C). Using left end probe B5T7/*Eco*RV. Lanes are the same as in (C).

186D7 clone is located 8.3 kb upstream of HS5, corresponding to position 29195 of AF137396 (GenBank). Recent research (27) showed that some odorant receptor genes are located upstream of the human β-globin gene locus. The BAC 186D7 clone does not contain the region, though both of the previous two YAC clones (8, 9) have the genes. The 3′ end of 186D7 clone is part of the interleukin-11 receptor α chain gene which is located on chromosome 9. To determine the composition of 186D7 clone, we performed FISH of human cells using the BAC186D7 DNA as the probe. Two kinds of signals were observed, one from the chromosome 11p15 region and the other from the chromosome 9p13 region (Fig. 3). The former should belong to the β-globin gene locus and the latter should belong to DNA sequences neighboring the interleukin-11 receptor α chain gene. In conclusion, the BAC186D7 clone contains the entire human β-globin gene locus, from the LCR to the adult β-globin gene and extends more than 20 kb 3′ to the β-globin gene. It also includes the 3′ HS site, which is found 20 kb downstream from the β-globin gene. At the same time, a DNA fragment of ~50 kb from chromosome 9p13 is located on the 3′ of the BAC clone.

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FIG. 3. Chromosomal location of BAC186D7 by fluorescent *in situ* hybridization (FISH). ▷ indicates that the signals came from two chromosomes.

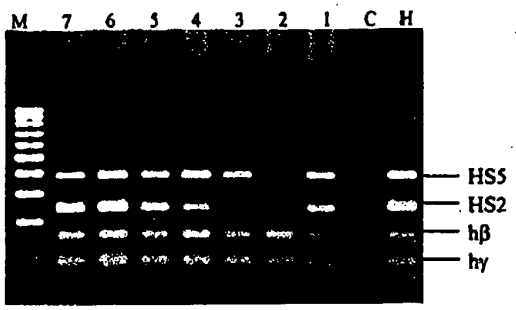

FIG. 4. PCR analysis of BAC transgenic founders. Line 1, β BAC38; line 2, β BAC60 line 3, β BAC68; line 4, β BAC69; line 5, β BAC86; line 6, β BAC90; line 7, β BAC91. C, negative control; H, human genomic DNA; M, 200-bp ladder marker. The four prime pairs used here are HS5, HS2, hγ, and hβ.

Generation and Characterization of BAC Transgenic Lines

The 186D7 BAC DNA was purified as a linear 160-kb fragment and microinjected into fertilized eggs to generate transgenic mice. Genomic DNA of founder mice was first analyzed by PCR using primer pairs from 5′HS5, 5′HS2 and the human γ- and β-globin genes (Fig. 4). Six founders (βBAC38, βBAC68, βBAC69, βBAC86, βBAC90, and βBAC91) which produced all the amplified fragments of expected size, and one founder (βBAC60) which only gave rise to the γ- and β-globin fragments, were selected from 148 pups. Only four lines (βBAC38, βBAC68, βBAC69, and βBAC60) were kept for further breeding. DNA from F1 and F2 mice was analyzed by Southern blot (Fig. 5) to confirm the structural integration of the β locus. The expected 10.5-kb *Eco*RI LCR, 6.9-kb *Eco*RI Aγ, 5.5-kb *Eco*RI β, and 1.0-kb *Eco*RI RK29 fragments were found in the βBAC38, βBAC68, βBAC69 lines, but only the 6.9-kb *Eco*RI Aγ, 5.5-kb *Eco*RI β, and 1.0-kb *Eco*RI RK29 fragments were found in the βBAC60 line which does not contain the LCR fragment. Using a PhosphorImager, the BAC copy number in these transgenic lines was estimated by comparing the β bands in transgenic DNA to the human genomic DNA of known amount. βBAC38 and βBAC68 contained single copy BAC inserts, whereas βBAC69 had two copies of the β-globin gene but only one copy of the LCR (data not shown). The above results indicate that the three transgenic lines each contain the entire LCR and all the β-like globin genes in one intact fragment. Line βBAC69 appears to carry another locus copy without the LCR. The map of the transgenic human β-globin locus in these lines is shown in Fig. 1.

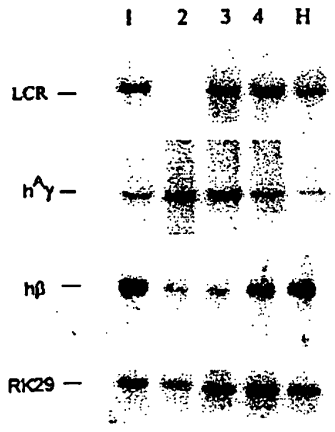

FIG. 5. Southern blot analysis of *Eco*RI-digested DNA prepared from BAC transgenic mice F1. The probes used in hybridization are HS4(A), hε (B), hβ (C), and RK29(D). For each, line 1, β BAC38; line 2, β BAC60; line 3, β BAC68; line 4, β BAC69; H, human genomic DNA.

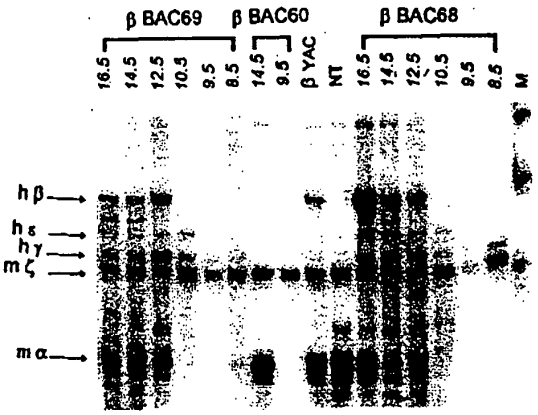

FIG. 6. Developmental expression of the transgenic human β-globin locus. RNase protection assay was used to analyze expression of human ϵ-, γ-, β-, and mouse ζ-, α-genes. Protected fragment sizes are as follows: human β (h β), 212 bp; human γ (hγ), 170 bp; human ϵ (h ϵ), 188 bp; mouse ζ (m ζ), 151 bp; mouse (mα), 122 bp. The developmental stage of prenatal samples is indicated above each lane. Lane NT, RNA from a nontransgenic mouse (18.5 day). Lane β YAC: RNA from a β YAC transgenic mouse (18.5 day).

Transcriptional Regulation of the Human β-Globin Genes in Transgenic Mice

The transcription of the human locus in transgenic mice was examined using RNase protection assay. First, expression of the human β-globin mRNA, but not ε-globin and γ-globin mRNA, was detected only in spleen and bone marrow samples of the transgenic mice (data not shown). Low-level expression of β-globin RNA was observed in the kidney and liver, which was probably caused by the residual blood in these tissues. These results showed that expression of the transgenic human β-globin gene is in a tissue-specific manner. Second, to estimate the developmental control of the β-like globin genes in the transgenic mice, F1 and F2 fetuses were obtained from timed pregnancies of lines βBAC68, βBAC69, and βBAC60. RNA isolated from these fetuses was analyzed by RNase protection assay (Fig. 6). At days 8.5 and 9.5, only the fetal γ-globin mRNA was detected in the cells of the yolk sac samples. In the 10.5-day fetus, the percentage of γ-globin mRNA decreased, whereas the ε-globin mRNA increased. At day 12.5, expression of the ε-globin gene had largely been shut down while the switch from γ to β globin gene expression occurred, coincident with expression of the endogenous murine ζ- to α-globin genes. As time passed, the human β-globin mRNA level continued to increase while the γ-globin mRNA level decreased at days 14.5 and 16.5. The expression levels of the human β-like globin genes were compared to that of the endogenous murine α-globin locus by PhosphorImager analysis. The expression pattern of globin genes in transgenic mice (Fig. 7) was drawn according to these data. At the same time, we could not detect any expression of the human globin gene in the βBAC60 transgenic line that lacked the LCR region. The amount of adult human β-globin mRNA compared with the endogenous murine α-globin in the four independent transgenic lines is listed in Table 1. For transgenic lines βBAC68 and βBAC69 we calculated the mean ± standard deviation (SD) from β-globin mRNA levels (corrected for copy number) in four F1 and F2 littermates. Three lines showed similar β-globin mRNA levels: βBAC38, 60.5%; βBAC68, 66.7% ± 5.6%; βBAC69, 68.7% ± 7.8%, although the fourth (βBAC60) had no detectable β-globin gene expression. The above data also indicated that the 5′ sequences present in the 248- and 150-kb β-YAC but missing in our BAC clone appear to not play an important role in globin gene expression and switching, although the region contains some newly characterized HS sites (27).

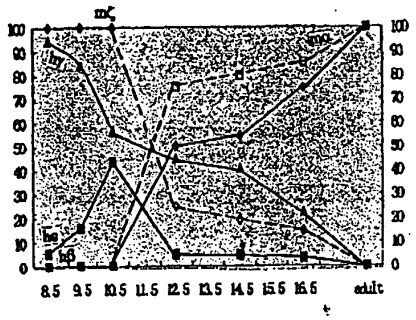

FIG. 7. Expression Pattern of human β-like globin genes in transgenic lines. RNA levels are the average of three transgenic mouse lines (βBAC38, βBAC68, and βBAC69). The RNA transcripts were quantitated with a PhosphorImage. The RNA level for each gene is calculated as the fraction of human β-like globin RNA or mouse α-like globin RNA. The developmental time was indicated at the bottom.

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TABLE 1

Characteristics of BAC-Mediated β-Globin Gene Locus Transgenic Mouse Lines

TA line	5′LCR	hβ and hγ gene	3′HS	hβ/mαmRNA (%)[a]
βBAC38	+	+	+	60.5
βBAC60	−	+	+	0.3
βBAC68	+	+	+	66.7 ± 5.6
βBAC69	+	+	+	68.7 ± 7.3

Note. Abbreviation used: TA, transgenic animal.

[a] Ten micrograms of total RNA from mouse spleen was performed using RNase protection analysis using human β and mouse α probes. The protected bands on the gel were quantitated via PhosperImage analysis. For lines βBAC68 and βBAC69, the mean ± standard deviation (SD) were calculated for four F1 and F2 littermates.

CE Analysis of Blood Samples from Transgenic Mice

CE was performed to confirm the presence of the human β-globin protein, which has an isoelectric point that is different from the endogenous mouse globin protein, in the peripheral blood cells of the transgenic mice. Compared with nontransgenic animal controls, CE of blood samples from transgenic mice carrying the β-globin gene locus all showed the significantly different peaks (Fig. 8), implying that the human β-globin gene is translated and the β-globin chains assemble with mouse α-globin chains to form hybrid hemoglobin tetramers.

DISCUSSION

The human β-globin gene locus has been extensively studied as a model system for understanding tissue- and developmental stage-specific expression of gene families. Previous transgenic studies have shown that the native order and sequence context of the β-globin gene locus are necessary for both appropriate timing and levels of expression (7–10). In the present study, we selected and purified a 160-kb BAC DNA carrying the entire human β-globin locus, and successfully introduced the BAC into mice. Several independent transgenic lines containing the entire β-globin gene locus were obtained. Furthermore, we showed that the expression of the transgenic human β-globin locus is tissue- and developmental stage-specific, and the expression level is similar among the three independent transgenic lines which carry the entire human β-globin locus. The expression pattern of human β-like globin genes in BAC-mediated transgenic mice is very similar, but not identical, to that of the endogenous murine globin genes. The existence of DNA fragments of chromosome 9p13 in BAC DNA didn't show any obvious influence on the expression of human β-globin genes in transgenic mice, which may be due to the integration position-independent expression of the entire human β-globin locus (11, 28). The human γ-globin gene is expressed earlier than the human ε-globin gene in the mouse, although γ-globulin genes are located farther from the LCR than the ε-globin gene. This phenomenon had also been observed in the β-globin gene locus transgenic mice mediated by both ligating cosmids and YAC (7, 8). This change of expressional order may be due to the different proximal regulatory elements between the γ- and ε-globin genes, and/or it could be related to the associated transcriptional factors in mice which are different from those in humans. Further experiments are required to determine the cause of this phenomenon.

The β-globin LCR, consisting of five DNase I hypersensitive sites, is necessary for high-level and integration position-independent expression of β-globin genes (11, 28). In general, HS2 acts as a classical enhancer for gene expression (29); HS3 is associated with the chromatin domain-opening function of the LCR (30), and HS5 exhibits enhancer-blocking activity characteristic of insulators (31). Transgenic studies have shown that deletion of the individual HS sites only mildly reduces the expression of the globin genes (11, 32, 33). Accumulating data have suggested that trans-acting factors bind to the HS sites to form a single LCR holocomplex that can then activate transcription of downstream genes through DNA looping (20, 30). In our data, no β-globin mRNA could be detected in the βBAC60 transgenic line which contained the β-globin genes and its proximal regulatory elements but not the LCR region. At the same time, the other three independent transgenic lines which

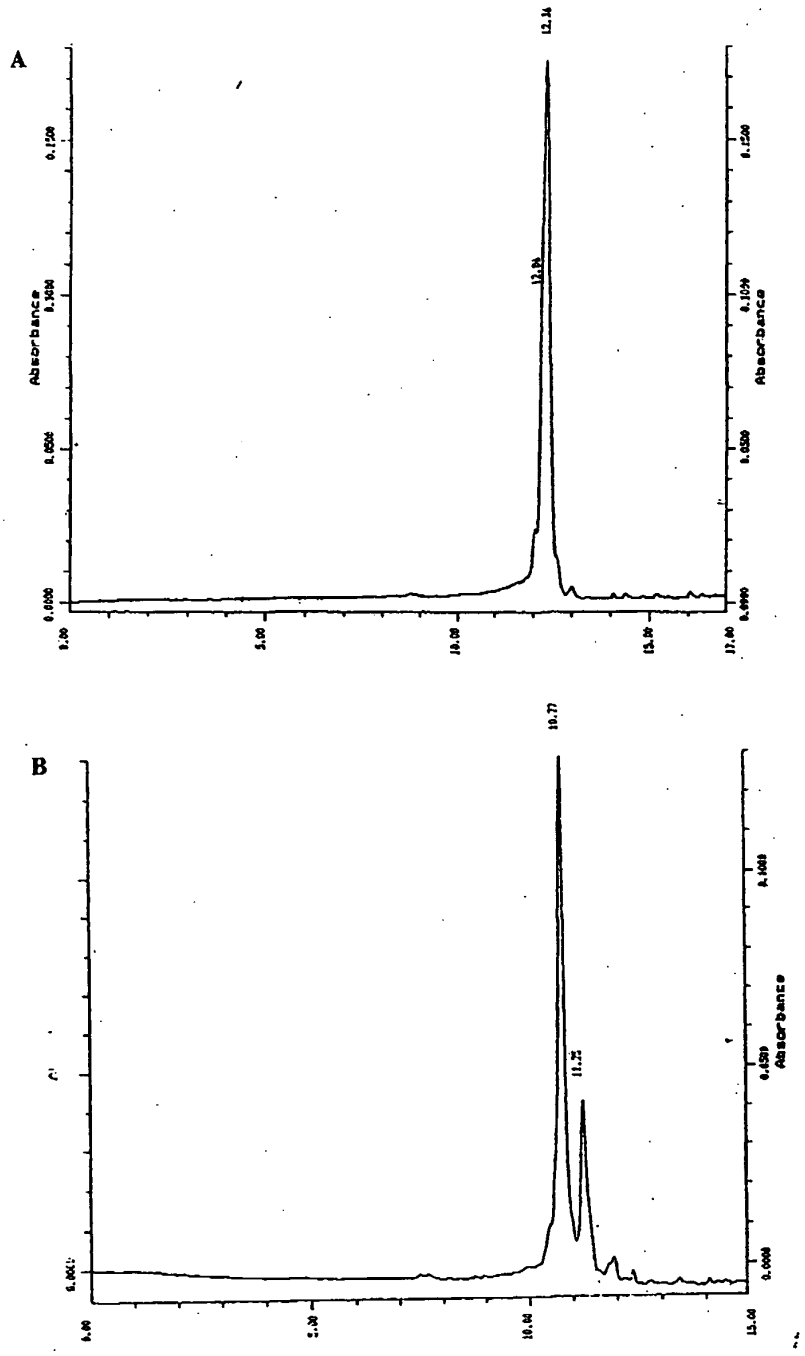

FIG. 8. Capillary electrophoresis analysis of peripheral blood of transgenic mice. (A) Negative mouse control; (B) BAC186D7 transgenic mouse.

contain the entire β-globin gene locus (including the LCR) showed the proper developmental expression, and the expression levels among the different transgenic lines are similar. The results suggest that the LCR can ensure correct β-globin gene expression in transgenic mice and that the β-globin BAC expression in transgenic mice can overcome the influence of the flanking chromosome environment of different integrated sites. Recently, Bulger *et al.* (27) have identified several

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new DNaseI HSs upstream from the HS5 of the LCR and shown that these structural features are evolutionarily conserved. Thus, our results indicate that the newly characterized HSs have no important function in β-globin gene expression and switching. Furthermore, the copy number of the BAC DNA integrated in the transgenic mice is very low (1 in two lines, 2 in two line). This property reduces the probability of DNA sequences integrating in tandem arrays, and creates expression artifacts or α/β-globin chain imbalance with potential adverse effects on the transgenic animals. All of these observations suggest that the transgenic mouse model system we have produced that is based on BAC and used to study the complex human β-globin gene cluster is stable and reproducible. This stability and reproducibility is the prerequisite for further studies for determining which kinds of *cis*-acting elements are included in regulating the developmental timing and the level of human β-globin gene expression. More recently, Kaufman *et al.* (34) have published their studies using β-globin BAC containing a 100-kb β-globin cluster DNA fragment partially digested from another 180-kb P1-derived artificial chromosomes (PAC) clone. They found that the expression level of human β-globin genes varies among different transgenic mouse lines (from 0 to 105%), and that $\gamma \rightarrow \beta$ globin gene switching is delayed to 18.5 days. The different results between their work and ours may be due to different contents of the two BAC clones.

The insertion of large foreign genomic DNA fragments into the mouse germline allows us to study the relationship between the structure and function of an entire gene locus and distant regulatory sequences. Large DNA fragment vectors such as cosmids (up to 50 kb) and YACs (average 500–600 kb) have been widely used. The BAC system, based on the *Escherichia coli* F factor, has been developed and can overcome these limitations (24, 35). First, BAC may propagate up to 300 kb foreign DNA, which is large enough to contain many gene clusters. Second, BAC has high stability and minimal chimerism. No rearrangements have been observed in it even after 100 generations of culture. Third, isolation and purification of intact BAC DNA is easier than cosmids or YACs because it exists as a supercoiled circular plasmid that can be handled in the same manner as conventional plasmids, allowing CsCl ultracentrifuge separation that results in the highly pure DNA samples necessary for microinjections. Compared with previous methods recovering linear DNA from agarose gel, separation of BAC DNA by CL-4B Sepharose columns can decrease both the dissolved microparticles in the DNA solution and the mechanical shearing. Presence of micro-particles affects the microinjection procedure while mechanical shearing affects the integrity of the transgene. In our Lab, linear 160-kb BAC DNA diluted in high-salt injection buffer (9) is stored at 4°C and no obvious degradation was detected in two months. Fourthly, several methods have been developed to modify BAC (36–38). Targeted modifications, such as deletion, substitution, point mutation and marker or reporter gene insertion, can all be easily performed. Recently, several research groups have used BAC as vector to establish the transgenic models for several gene clusters, including the mouse C*lock* gene locus (39), human apoB gene locus (40), mouse myosin gene(41) and human zinc-finger transcriptional factor *Ziprol* gene (42). Our results demonstrate that BACs can be used to perform research work with ease and reproducibility. Analysis of β-globin BAC transgenic mice will provide insights into long-range regulatory mechanisms of gene loci. In addition, β-globin BAC transgenic mice are a good model system for human genetic diseases, such as β-thalassemia and hemoglobinopathies because they can be used to analyze the regulation of abnormal genes, thus providing preclinical data for human gene therapy.

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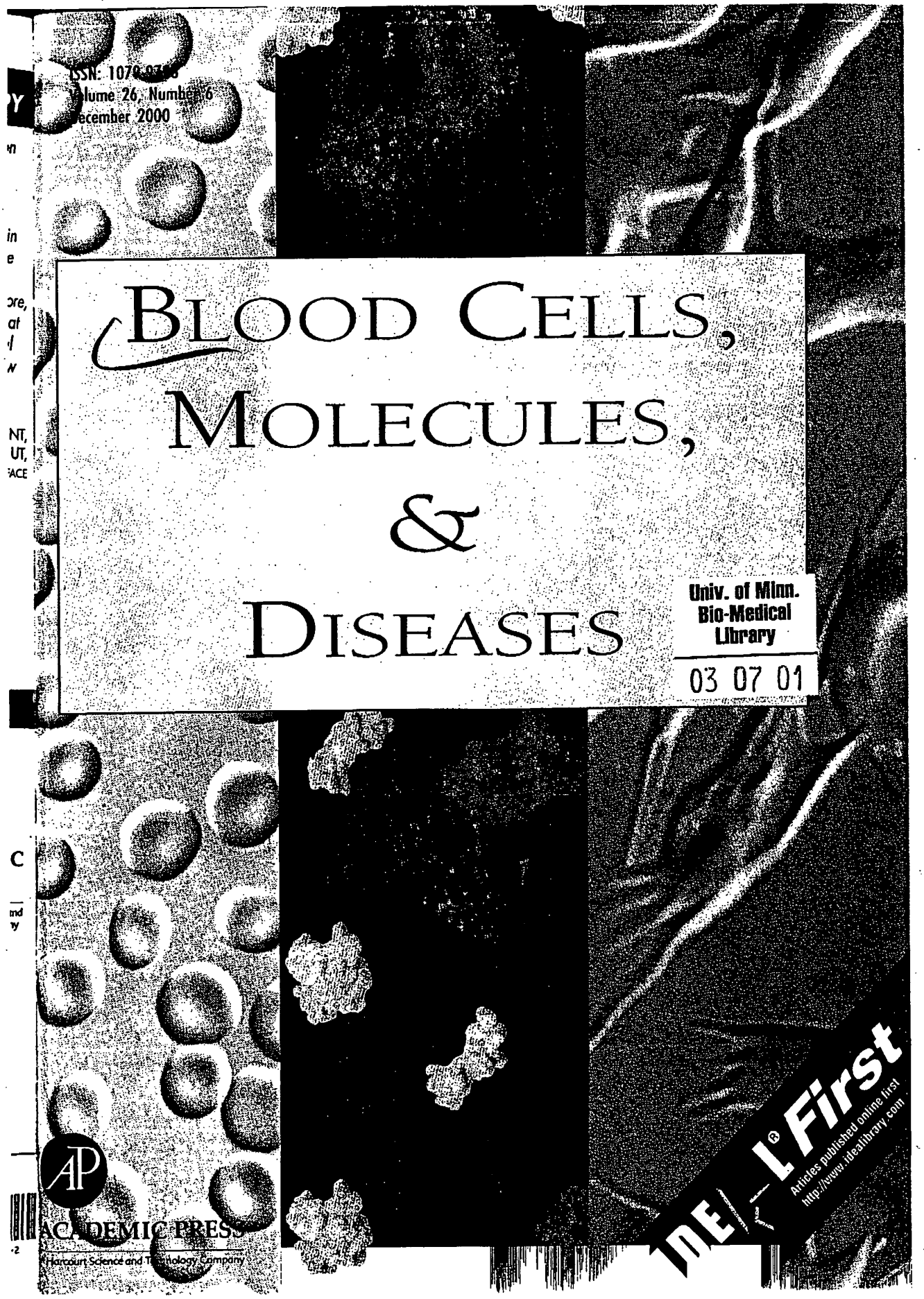
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